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Development and Evaluation of Multiple Regression Model for Prediction of Carcinogenic Risk

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ABSTRACT

The internal dose of Po-210 in marine organisms collected from a coal burning power plant area, has been calculated in this study. On the other hand, risk assessment, due to the intake of Po-210 and toxic of heavy metals via the consumption of seafood product has also been evaluated. Alpha Spectrometry and Inductively Coupled Plasma Mass Spectrometry techniques were used to determine the concentration of Po-210 and heavy metals, respectively, which were used to calculate the carcinogenic risk and heavy metal risk. The calculated mean values of weighted average internal dose rate from internal exposure of Po-210 were 0.061±0.028, 0.197±0.137 and 1.645±1.521 μGyh^{-1} for *Arius maculatus*, *Penaeus merguiensis* and *Anadara granosa,* respectively. The findings of this study indicate a potential health risk due to the consumption of seafood collected from coal burning power plant area. However, Cd and Pb have been identified as the predictor for carcinogenic risk because of the intake of Po-210.

Keywords: Health risk, Po-210, heavy metal, carcinogenic, dose.

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INTRODUCTION

Seafood and their products are considered to be one of the major sources of protein for coastal public and have high export significance. Marine organisms have the capability of accumulating radionuclides and toxic elements from water, and that is why the determination of radioactivity and toxic metals in marine food supplies like crustaceans, molluscs and fishes presumed to be greater importance measurement of natural background levels and estimation of dose to public in terms of health safety is essential, since these values form reference values for comparing radionuclide concentration due to anthropogenic activities [19]. Among the various radionuclides occurring in the marine environment, Po-210 assumes greater importance because of their high accumulation potential, especially in seafoods. However, Po-210 is known to be the major contributor (90%) to the natural radiation dose coming from alpha emitting radionuclides received by most marine organisms [7, 20]. On the other hand, seafood, part of being a good source of digestible protein, vitamins, minerals and polyunsaturated fatty acid, are also an important source of heavy metal. Usually, living organisms require trace amounts of some heavy metals but could be very harmful when present at excessive concentration.

As Malaysia is among the countries with highest fish consumption in the world, information on the intake of radionuclides and toxic heavy metals through seafoods is important for the risk assessment. In the present study, the internal dose has been calculated. At the same time the carcinogenic risk due to the intake of Po-210 has been predicted by the assessment of heavy metal risk for the consumption of seafood collected from Kapar coastal area where a mega coal burning power station is operating and is supposed to be the first initiative in this regards.

MATERIALS AND METHODS

Sampling

The organism samples were collected from the local fish market, which is very near to Sultan Salahuddin Abdul Aziz power plant (Figure 1). Samplings were carried out on August 2008, December 2008 and February 2009. Three types of marine organisms such as *Arius maculatus*, *Penaeus merguiensis* and *Anadara granosa* have been selected for this analysis. During each sampling period, 30 individuals of each species were collected to reduce the individual variation in Po-210 accumulation and 15 samples were used for the analysis of heavy metals. The organism samples were transported to the laboratory for further analysis and kept in freezer.

Sample preparation

For the preparation of samples, only edible parts were selected. The soft tissues and muscles from the shells and bones have been separated from molluscs, crustaceans and fishes. The wet weights of the samples have been recorded and then dried in an oven at 60^o C overnight to obtain the dry weights. After drying, mass of the dried samples was determined and the fresh weight to dry weight ratio has been calculated. Then, the samples were homogenized with a mortar. Finally, the samples were wrapped with aluminium foil and preserved in leveled plastic bag for radiochemical and heavy metals analysis.

Figure 1. Study area showing the fish market at Kapar coastal area.

Analysis of Po-210

The radiochemical separation method was used to estimate Po-210 in the samples [2, 12]. About 0.5 g of the dried sample was taken and Po-209 of a known activity was added as a yield tracer. Then the samples were digested with nitric acid and perchloric acid. The solution was filtered and gently evaporated to dryness. Then the samples were dissolved in 50 ml of 0.5 M HCl along with a pinch of ascorbic acid to reduce Fe (III) and Po-210 was spontaneously deposited on brightly polished silver discs (2 cm diameter) for a period of 3-4 hours at a temperature of 70-90ºC.

The discs were counted for Po-210 activities with an alpha spectrometry system. The extraction yield varied from 80 to 95% for the organism samples. Additionally, the combined standard uncertainty of 2σ was calculated involving all the sources of uncertainty. The Po-210 deposition was carried out within 2 months of sampling and the activities were calculated at the date of sampling. To ensure the quality of the methodology, Po-210 was estimated in a

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certified reference material IAEA-134 (Cockle flesh) and the measured values were under the 95% confidence interval.

Analysis of heavy metals

Three replicates of organisms sample were analyzed for the measurement of heavy metals such as Cu, Cd, Zn, Pb and Cr, and all the glass wares used for analysis were acid washed to avoid the possible contamination. About 0.3-0.5 g of dried samples for each replicate were weighted in a beaker using electronic scales. The samples were then digested with a mixture of 30 ml nitric acid (HNO_3; GR, 65%, Merck) and 5 ml of concentrated perchloric acid ($HClO_4$; GF; 70%, Merck). After that, 10 ml of concentrated hydrochloric acid (HCl, GR; 37%, Merck) was added in the samples and heated until dryness. After cooling the sample, 2.5 ml of nitric acid was added into the samples. A total of 20 ml of de-ionized distilled water was added into the beaker containing the sample and filtered through filter paper (Whatman, GF/C; diameter 47 mm; pore size 0.45 µm). After that, the filtered solutions were added with de-ionized distilled water until 70 ml to make it to 0.5M HNO_3. Determination of heavy metals was carried out using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer-Elan 9000). In this study SRM-407 provided by the International Atomic Energy Agency was used for marine organisms. The analytical results for the investigated heavy metals in reference material were within or near the certified values and the recoveries of all the metals ranged between 81.29 to 88.89%.

RESULTS AND DISCUSSION

Internal dose calculation of Po-210

In this study, we have calculated the ''weighted'' internal dose due to Po-210 received chronically by the marine species using a simplified approach based on the equation and parameters (DCC) recommended by the FASSET program [23]. The calculation equation is:

$$D = A \times DCC \quad \text{................. (i)}$$

Where D = dose rate from internal exposure of Po-210 (μGyh^{-1}); A = Activity concentration of Po-210 ($Bqkg^{-1}$w.w) and DCC = Dose Conversion Coefficient, internal (μGyh^{-1} per $Bqkg^{-1}$).
For our calculations, for the DCC we took the value 3.1×10^{-2} μGyh^{-1} per $Bqkg^{-1}$ according to (Prohl; Vives i Batlle *et al.*).

On the basis of our measurement the weighted average internal dose rate from internal exposure varies from 0.002 to 0.175 μGyh^{-1} (mean 0.061±0.028 μGyh^{-1}) for *Arius maculatus*. In *Penaeus merguiensis*, the internal dose rates varied from 0.02 to 0.952 μGyh^{-1} (mean 0.197±0.137 μGyh^{-1}) while in *Anadara granosa* the value is much higher, i.e. 0.143 to 7.431 μGyh^{-1} (mean 1.645±1.521 μGyh^{-1}). Brown et al. [4] estimated that the total dose due to natural radionuclides (^{40}K, ^{210}Po, ^{226}Ra, ^{228}Ra, ^{228}Th, ^{230}Th, ^{232}Th, and ^{238}U) absorbed by molluscs is 1.52 μGyh^{-1} which is slightly lower than that of present study. Besides that, the calculated values of present study corresponds to the values of previous studies [6,9]. At the present time, the development of a system ensuring the protection of the environment against ionising radiation is internationally debated [15, 17, 22]. Therefore guideline values, such as the PNEDR (Predicted-No-Effect Dose Rate) i.e. the potential value (10 μGyh^{-1} for aquatic ecosystems) below which it is accepted that a chronic dose has no effect at the population level, have been recently derived [13]. However, the internal exposure values, which we calculated to be between 0.002 and 7.43 μGyh^{-1} for the analyzed species, can be compared with the PNEDR (Predicted No Effect Dose Rate) value of 10 μGyh^{-1} and our calculation indicates that the contribution of Po-210 only to chronic irradiation may be nearly 37% of this value. But Connan et al.calculated the contribution of Po-210 on aquatic organisms about 10% of PNEDR value. However, it is therefore unexpected that Po-210 concentrations observed in the analyzed species lead to high radiation exposure.

Multiple regreation model for the Prediction of carcinogenic risk

In *Arius maculatus* the concentration of Po-210 ranged from 0.06 to 5.64 Bqkg^{-1} (fresh weight) with the mean value of 1.93±0.91 Bqkg^{-1} (fresh weight). At the same time, the concentration ranged from 0.65 to 30.72 Bqkg^{-1} (fresh weight) in *Penaeus merguensis* where the mean value was 6.37±4.44 Bqkg^{-1} (fresh weight). On the other hand, in case of *Anadara granosa* the activity concentration of Po-210 varied between 4.61 and 239.72 Bqkg^{-1} (fresh weight) with the mean value of 53.09±49.08 Bqkg^{-1} (fresh weight).

However, risk is the probability of harmful effect on a human. The alpha particles emitted by Po-210 only travel a very short distance in the air. As a result only presents a health risk in the event of internal contamination or direct contact with the skin. The lifetime cancer risk due to the intake of Po-210 via seafood consumption, R, was calculated according to the following equation,

$$R = r \times I \text{ [1] -------------- (ii)}$$

r is the cancer risk coefficient and I is the average lifetime intake of Po-210. Taking into account the average Malaysian life expectancy at birth of 73.39 years, [5] the life time intake of Po-210 via seafood consumption was calculated from the daily intake. The mortality cancer risk coefficients of Po-210 was 4.44×10^{-8} riskBq^{-1} [11].

The mean mortality risk calculated 0.24×10^{-3}±0.11×10^{-3} for *Arius maculatus*, 0.77×10^{-3}±0.54×10^{-3} for *Penaeus merguiensis* and 6.57×10^{-3}±6.01×10^{-3} for *Anadara granosa*. Usually, cancer risks that range between 1×10^{-6} and 1×10^{-4} are considered to be acceptable [11, 14]. However, the concept of 10^{-6} is controversial as it is concluded that despite its widespread use: no agencies could provide documentation on the origins of 10^{-6} as acceptable risk level [18]. Therefore, in case of present study, we have compared the calculated values of cancer risk with the ICRP cancer risk factor of 2.5×10^{-3} which is based on the additional annual dose limit of 1 mSv for general public [16, 19]. In this case the calculated values of cancer risks for *Arius* and

Penaeus are lower than the risk factor whereas the calculated values are much higher in case of *Anadara*.

The mean metal concentration of muscles of three types of seafood analyzed in this study was used to conduct the health risk assessment and the calculated values are presented in Table I. Risk assessment of chemicals is the practice of evaluating the risk associated with chemical exposure which determines the kind and degree of hazard posed by a chemical and thereby permits an estimate of the present of potential risk. For the risk assessment process, the estimated total doses were compared with doses considered by the USEPA to be safe for a person to receive every day for a life time which is called reference dose (RfD). Reference doses take into account that toxicity can accumulate in the organism when the chemical is received as frequently as everyday. The comparison was made by calculating the hazard quotient (HQ), which is the person's total dose divided by the RfD. Among the heavy metals analyzed in this study, the USEPA developed the RfD values for Cr, Cd, Cu and Zn [25]. In case of Pb, the provisional tolerable daily intake of 3.57 μg/kg body weight/day was used [10, 24]. Doses below the RfD yield HQ less than 1 and those greater than the RfD yield HQ greater than 1. If the calculated dose is equal to the safe dose (RfD), then the HQ is 1 [3]. The hazard index (HI) was treated as the mathematical sum of each individual element HQs for three types of seafood analyzed [8].

Table I. Calculated values of heavy metals concentrations, HQ and HI in analyzed species.

Species	Heavy metals	$\mu g g^{-1}$ (ww)	HQ	HI
Arius maculatus	Cd	0.14	0.29	1.90
	Cu	1.21	0.06	
	Zn	41.84	0.29	
	Pb	1.50	0.87	
	Cr	0.55	0.39	
Penaeus merguiensis	Cd	0.1	0.21	1.32
	Cu	12.06	0.03	
	Zn	42.41	0.29	
	Pb	1	0.58	
	Cr	0.29	0.2	
Anadara granosa	Cd	0.82	1.71	3.38
	Cu	3.39	0.18	
	Zn	51.63	0.36	
	Pb	0.97	0.57	
	Cr	0.8	0.56	

The risk assessment (HQ) due to toxic heavy metals was calculated according to [21] using the estimated daily intake (EDI) and reference dose (RfD) and calculated by the following equation:

$$HQ = EDI/RfD \quad \text{......... (iii)}$$

In case of *Arius maculatus* the HQ has been calculated as 0.29, 0.06, 0.29, 0.87 and 0.39 for Cd, Cu, Zn, Pb and Cr, respectively. The HQs calculated for *Penaeus merguiensis* were, 0.21, 0.03, 0.29, 0.58 and 0.2 for Cd, Cu, Zn, Pb and Cr, respectively, whereas in *Anadara granosa* the HQ values were 1.71, 0.18, 0.36, 0.57 and 0.56 for Cd, Cu, Zn, Pb and Cr, respectively. The HI values in all the organisms are ranging from 1.32 to 3.38 which greater than 1. As the calculated HI values of this study are greater than 1, it can be assumed that the potential risk is existed.

However, this research utilizes a multiple regression model to explore the effect of toxic heavy metals on the risk of Po-210. Using SPSS, a multiple regression model was developed relating the mortality risk of Po-210 (the dependent variable that is being predicted) with the risk of heavy metals (the predictor variables). The normality of the data set has been checked using Shapiro-Wilk's test. As the data set demonstrated asymmetric distribution, all values were transformed to square root.

Table II Model Summary of multiple regression

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.708	0.502	0.482	0.02475

Table III Results of ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.080	5	0.016	25.977	0.000
	Residual	0.079	129	0.001		
	Total	0.159	134			

Table IV Results of the Multiple Regression Analysis for the prediction of carcinogenic risk of Po-210 due to consumption of seafood.

Variables	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	0.055	0.014		3.954	0.000
Cd	0.057	0.008	0.730	7.005	0.000
Cu	-0.042	0.030	-0.168	-1.425	0.156
Zn	-0.001	0.018	-0.004	-0.067	0.947
Pb	-0.059	0.011	-0.336	-5.170	0.000
Cr	0.002	0.016	0.012	0.133	0.895

a Dependent Variable: Po-210

The model summary and the results of the analysis of variance (ANOVA) for the regression model are shown in Table II and Table III respectively. The p-value (significance) for the model was almost zero, indicating the regression model is significant. The R-squared for this model was 0.502, indicating that the model has accounted for almost 50 percent of the variance in the criterion variable. Since the model proved to be significant and the strength of association between the dependent and the independent variable was rather high, it was used for prediction.

The results of the regression analysis are shown in Table IV, giving the following regression model:

Y	=	0.055 (0.000)	+	$0.057X_1$ (0.000)	-	$0.042\ X_2$ (-0.156)	-	$0.001X_3$ (-0.947)	-	$0.059X_4$ (-0. 000)	+	$0.002\ X_5$ (0. 895)

Where,
y = Response variable (Mortality risk of Po-210)
x_1 = Controlled variable (Cd)
x_2 = Controlled variable (Cu)
x_3 = Controlled variable (Zn)
x_4 = Controlled variable (Pb)
x_5 = Controlled variable (Cr)

As can be seen, only two independent variables were found to be significantly influential across all data periods which are Cd and Pb. Thus it is concluded that the calculated risk of Cd and Pb can be the predictor for the carcinogenic risk due to the consumption of seafood product.

CONCLUSION

In this study, the internal exposure values for the analyzed species have been compared with the Predicted No Effect Dose Rate value and our calculation indicates that the contribution of Po-210 only to chronic irradiation may be nearly 37% of this value. From the findings it can be concluded that the population of the coal burning area is subjected to a higher alpha radiation exposure and higher heavy metal toxicity from the consumption of seafood as the risk levels are higher. However, the findings of multiple regression analysis indicated that the calculated risk of Cd and Pb can be the predictor for the carcinogenic risk due to the consumption of seafood product. Additionally, this study proved that the multivariate statistical analysis can be the effective tool in the field of radiochemical research.

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